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Discarded Placentas Deliver Researchers Promising Cells Similar To Embryonic Stem Cells

08/05/05 – Routinely discarded as medical waste, placental tissue could feasibly provide an abundant source of cells with the same potential to treat diseases and regenerate tissues as their more controversial counterparts, embryonic stem cells, suggests a University of Pittsburgh study to be published in the journal Stem Cells and available now as an early online publication in Stem Cells Express.

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A part of the placenta called the amnion, or the outer membrane of the amniotic sac, is comprised of cells that have strikingly similar characteristics to embryonic stem cells, including the ability to express two key genes that give embryonic stem cells their unique capability for developing into any kind of specialized cell, the researchers report. And according to the results of their studies, these so-called amniotic epithelial cells could in fact be directed to form liver, pancreas, heart and nerve cells under the right laboratory conditions.

"If we could develop efficient methods that would allow amnion-derived cells to differentiate into specific cell types, then placentas would no longer be relegated to the trashcan. Instead, we'd have a useful source of cells for transplantation and regenerative medicine," said senior author Stephen C. Strom, Ph.D., associate professor of pathology at the University of Pittsburgh School of Medicine and a researcher at the university's McGowan Institute for Regenerative Medicine.

According to U.S. census figures, there are more than 4 million live births each year. For each discarded placenta, the researchers calculate there are about 300 million amniotic epithelial cells that potentially could be expanded to between 10 and 60 billion cells relatively easily.

"Provided that research advances to the point that we can demonstrate these cells' true therapeutic benefit, parents could conceivably choose to bank their child's amniotic epithelial cells in the event they may someday be needed, as is sometimes done now with umbilical cord blood," commented Dr. Strom.

The amnion is derived from the embryo and forms as early as eight days after fertilization, when the fate of cells has yet to be determined, and serves to protect the developing fetus. According to the researchers' studies using placentas from full-term pregnancies, amniotic epithelial cells have many of the telltale surface markers that define embryonic stem cells, and also express the Oct-4 and nanog genes that are known to be required for self-renewal and pluripotency ? the ability to develop into any type of cell.

Yet the authors are careful to point out that despite their remarkable similarities to embryonic stem cells, amniotic epithelial cells are not stem cells per se, because they can't grow indefinitely. This may be due to the fact that these amnion-derived cells do not express a certain enzyme, called telomerase, that is important for normal DNA and chromosome replication, and by extension, ultimately, cell division.

"Perhaps it's to their advantage that the amnion epithelial cells lack telomerase expression, because telomerase is associated with many cancers and one of the main concerns about stem cell therapies is that transplanted stem cells would replicate in the recipient to form tumors," noted Toshio Miki, M.D., Ph.D., first author of the paper and an instructor in the department of pathology at the School of Medicine.

To help determine if amnion-derived cells that are delivered directly to tissues would cause tumors, the researchers conducted studies in immune system-deficient mice and found no evidence that tumors had developed seven months after the cells were injected into multiple sites.

While amniotic epithelial cells do not share the same capacity for unlimited replication as do embryonic stem cells, they still can double in population size about 20 times over without needing another cell type serving as a feeder cell layer. This is significant, because to replicate, the currently available embryonic stem cell lines require a bed of mouse cells, traces of which can end up in each new generation of stem cells. Amniotic epithelial cells, on the other hand, create their own feeder layer, with some cells choosing to spread out at the bottom of the culture dish thereby giving those cells just above them the best environment for replicating and for retaining their stem cell characteristics.

With the addition of various growth factors, the authors report the amnion-derived cells could differentiate to become liver cells, heart cells, the glial and neuronal cells that make up the nervous system, and pancreatic cells with genetic markers for insulin and glycogen production.

"In this first paper we sought to determine if amniotic epithelial cells have the potential to differentiate into many different cell types rather than focusing on ways for optimizing this potential for a specific cell type. Further studies will be required to better understand if and how they may be useful in a clinical setting," Dr. Strom added.

The researchers say their original motivation was, and still is, to identify cells with the same therapeutic promise as embryonic stem cells. To this end, they began looking at the viability of amnion as a cell source in late 2001, obtaining discarded placentas from full-term births under an Institutional Review Board-approved protocol. In 2002, the University of Pittsburgh licensed the technology to a company now called Stemnion, LLC, and as part of the agreement, and in keeping with university patent policy, Drs. Strom and Miki will receive license proceeds. Both have served as paid consultants and hold equity in Stemnion.

The research was supported by the Alpha-1 Foundation and the National Institute of Diabetes and Digestive and Kidney Diseases, a part of the National Institutes of Health. In addition to Drs. Miki and Strom, other authors are Thomas Lehmann, Ph.D., and Hongbo Cai, M.D., Ph.D., both from the department of pathology, and Donna Stolz, Ph.D., of the department of cell biology and physiology.

Source: University of Pittsburgh Medical Center

Unipotent cell

From Wikipedia, the free encyclopedia.

Unipotent cell, in cell biology, is used to describe a cell (e.g. a stem cell) which has the capacity to develop/differentiate into only one type of tissue/cell type.

Retrieved from "http://en.wikipedia.org/wiki/Unipotent_cell"

Category: Cell biology

From Bench to Bedside

Stem cell therapies: a tale of caution

Edward Byrne and David W Howells

MJA 2003; 179 (3): 164-166

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Abstract

- One of the most exciting possibilities in human therapeutics is that stem cells (embryonic or adult) may compensate for cell loss in disease, with functional recovery.
- This has received considerable publicity in the lay press.
- Much work remains to be done to turn stem cell therapy into a practical reality for major degenerative diseases, especially those affecting the nervous system.
- Medical scientists and journalists should work together in ensuring that the general public has a realistic understanding of the likely time frame in which benefits from stem cell therapies will be realised.

ONE OF THE CHALLENGES facing modern medical science is to develop the means to regenerate failing organs in incurable illness. This applies especially to the central nervous system (CNS); many of the major CNS illnesses have either no treatment or relatively ineffective treatments.

In recent years in Australia, advances in stem cell technologies have claimed a higher public profile than any other medical advance. This has fostered a public perception that new treatments for disorders such as stroke, Parkinson's disease, Alzheimer's dementia and motor neurone disease, are not only highly likely, but imminent. Australia has outstanding groups working in both the adult and embryonic stem cell technologies. As a result, scientific advances often receive a high media profile for

work which is still some distance from clinical trials, let alone translation into proven remedies. Indeed, the correspondence columns of major newspapers and the current embryonic stem cell debate reveal a high expectation by some members of the public that stem cell therapy will soon provide successful treatments for progressive neurodegenerative disease. Further, anecdotal information suggests that the potential benefits of stem cell therapy are now commonly discussed in clinical practice.

The topicality of stem cell research is indicated by 93 articles which mention stem cells in one newspaper, *The Melbourne Age*, over the past 12 months. Topics covered include an article indicating that stem cell therapies may be expensive and only available to the wealthy, and articles linking stem cell research to treatments in AIDS, renal disease and cardiac failure. One recent report in the electronic media mentions stem cells as a possible future treatment for multiple sclerosis. It is clear that the non-scientific or non-medical reader could justifiably conclude from much of this information that practical stem cell therapies for many human conditions will be available in the not-too-distant future.

Types of stem cells

There are many different types of stem cells. Cells from fertilised ova form embryonic stem cells, which can develop into any tissue or tissues. Stem cells found in most adult tissues still have the potential to generate several different types of tissue. Other stem cells are already advanced on a particular lineage and are committed to one cell type (unipotent).[1]

One of the keys to most approaches to stem cell therapy is to generate a stable pool of cells. These are usually unipotent. However, in some cases, such as the CNS, several cell lines (ie, neuronal, glial) might be required at the same time for clinical applications. Skeletal muscle is, in many ways, an ideal tissue for stem cell therapy, in that it has been shown that the muscle architecture can be restored even after severe disruptions.[2] Endogenous muscle cells are known to be exhausted in degenerative muscle disease such as Duchenne dystrophy.[3]

Work with muscle cells

Attempts to reconstitute skeletal muscle in human muscle disease through myoblast transfer therapy (a form of unipotent stem cell therapy) have now been under way for more than a decade, and provide many lessons about the hurdles that have to be overcome in stem cell therapy. Although early experiments in animal models of Duchenne dystrophy showed that transplantation infusion of committed muscle stem cells from normal muscle resulted in partial restoration of the deficient protein, a high percentage of introduced stem cells died within a short time of transplant, and clinical trials in boyhood Duchenne dystrophy showed no significant clinical improvement.[4]

Ten years of work with a fairly simple stem cell model in a non-complex tissue that can regenerate effectively has been frustratingly slow. Major problems of immunorejection, both of foreign cells and dystrophin, have not been adequately overcome with readily available immunosuppressive therapies. Difficulties in delivering stem cells to a wide range of muscle tissues remain formidable, and, in spite of enormous hope and enthusiasm for this strategy by investigators and the muscular dystrophy community when it was introduced, little practical progress has been made.

Problems with stem cell therapy for the central nervous system

The complexity of issues and the range of problems to be overcome in achieving stem cell therapy for the CNS dwarf those in skeletal muscle.[5] These problems include:

- isolation, enrichment and propagation of stable CNS neural stem cell lines are not yet reliable, although this field is advancing rapidly;
- processes which allow introduced stem cells to help restore injured neuronal networks in the damaged adult brain are not yet adequately understood;
- disease processes in progressive neurological disorders which may adversely affect introduced stem cells need to be understood and alleviated;
- factors that drive introduced stem cells preferentially to glial lines in most parts of the CNS need to be better understood and able to be manipulated;
- there are delivery problems in generalised CNS disorders because of a need to deliver stem cells to many different parts of the brain or spinal cord (this applies to Alzheimer's dementia and motor neurone disease);
- introduced stem cells must have a useful physiological role, and neurones must be integrated into effective neuronal networks — those that are not could theoretically impair function (similar considerations apply to other tissues, such as heart, where stem cell therapy is only likely to be useful if generated cardiomyocytes are effectively incorporated into the contracting myocardium);
- uncontrolled proliferation of stem cells can result in benign tumours (teratomas), which may be of great significance in the CNS (and the myocardium).[5]

Other developments

Exciting developments in bone-marrow-derived stem cell technology have the potential to overcome some of these difficulties. The identification of pluripotent cells in the two major bone marrow fractions (the mesenchymal and haemopoietic cell fractions), which can differentiate into a number of cell lines, offers a potential for the

development of treatments that can be delivered systemically and manipulated to avoid rejection.[6] Treating three children who had osteogenesis imperfecta with allogenic bone marrow transplantation allowed mesenchymal progenitor cells to differentiate into osteoblast lineages, resulting in new bone formation and clinical improvement.[7] This approach has potential in a number of tissues, but currently the ability of mesenchymal stem cells to repopulate tissues such as muscle is low.

In stroke studies in which millions of cells were implanted into animals, as few as 330 of the implanted cells took on any role, and implantation did not reduce infarct volumes.[8] Thus it seems likely that these stem cell implants stimulate endogenous host repair mechanisms[8] or provide a degree of neuroprotection which limits the effects of ongoing damage rather than replace lost neurones and repair the damaged neural architecture.

Ongoing work is also necessary to further define the growth potential of stem cell subtypes, and to identify the factors that drive differentiation in a particular direction. In the neurological sphere, most work has been done on Parkinson's disease. This condition lends itself to stem cell therapy in that the targeted area is small and accessible neurosurgically in a part of the brain that neurosurgeons are familiar with through a long history of stereotaxic surgery.

When fetal dopaminergic tissue (not stem cells) is implanted into the striatum of patients with Parkinson's disease, it normalises dopamine turnover and has produced a moderate clinical benefit which develops gradually over 6–24 months.[9-12] However, in a double-blind trial of fetal dopaminergic cell transplantation, which included a sham surgery arm, the results fell short of those expected from previous case reports and short series.[13] Although postmortem examination of two patients showed marked improvements on ^{18}F-fluorodopa positron emission tomography scans and dopaminergic re-innervation of the putamen,[13] only modest clinical improvements were seen in younger patients (aged < 60 years), and older patients showed no overall improvement. Importantly, 15% of these patients developed severe dystonia and dyskinesias that persisted even when levodopa therapy was ceased. While they may provide a more convenient source of tissue, there is no conceptual framework to suggest that survival, differentiation and integration of stem cells will lead to a better outcome than survival and integration of predifferentiated fetal mesencephalic dopaminergic neurones. It should also be noted that when embryonic stem cells were implanted into the denervated striatum of hemiparkinsonian rats, 20% of animals developed teratomas.[14]

The road ahead

Stem cell therapy holds huge promise, not only for brain disease, but also for many other illnesses that are currently incurable. The science in this area is currently in the early stages of development, and a huge amount of work and new discovery is a prerequisite to realising these hopes. It is not known which degenerative disorders will

be treatable by stem cell therapies, or how long the work will take. Information about scientific achievements needs to be communicated to the public properly, and in a rigorous and cautious way, so that it does not raise excessive expectations.[5] Further, confining the hopes of a cure for neurological disease to stem cells is reductive and possibly even risky. Stem cell therapy will be important, but probably not sufficient in itself to treat neurodegenerative disease.[5]

The great majority of neuroscientists, including those working in the stem cell area, would probably agree with these views. The current explosion in new knowledge in neurobiology offers real hope in many areas. However, it is crucial that the medical–scientific and medical–media communities work together to keep the general public not only well informed, but also realistically appraised as to the significance of scientific breakthroughs in the development of new treatments.

Competing interests

None identified.

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Medical Dictionary

83 entries found for **factor**. Select an entry and then click 'Go'.

factor
animal protein factor
antianemic factor
antihemophilic factor
atrial natriuretic factor
chill factor

Go

Main Entry: **fac·tor**
Pronunciation: 'fak-tər
Function: *noun*
1 a : something that actively contributes to the production of a result b : a substance that functions in or promotes the function of a particular physiological process or bodily system
2 : GENE
- **fac·to·ri·al** /fak-'tōr-ē-əl, -'tȯr-/ *adjective*

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Pronunciation Key

\ ə \ as a in abut	\ g \ as g in go	\ r \ as r in red
\ 'ə ɪə \ as u in abut	\ h \ as h in hat	\ s \ as s in less>
\ ᵊ \ as e in kitten	\ i \ as i in hit	\ sh \ as sh in shy
\ ər \ as ur/er in further	\ ī \ as i in ice	\ t \ as t in tie
\ a \ as a in ash	\ j \ as j in job	\ th \ as th in thin
\ ā \ as a in ace	\ k \ as k in kin	\ th̲ \ as th in the
\ ä \ as o in mop	\ k̲ \ as ch in ich dien	\ ü \ as oo in loot
\ aů \ as ou in out	\ l \ as l in lily	\ ů \ as oo in foot
\ b \ as in baby	\ m \ as m in murmur	\ v \ as v in vivid
\ ch \ as ch in chin	\ n \ as n in own	\ w \ as w in away
\ d \ as d in did	\ ŋ \ as ng in sing	\ y \ as y in yet
\ e \ as e in bet	\ ō \ as o in go	\ yü \ as you in youth
\ 'ē ɪē \ as ea in easy	\ ȯ \ as aw in law	\ yů \ as u in curable
\ ē \ as y in easy	\ ȯi \ as oy in boy	\ z \ as z in zone
\ f \ as f in fifty	\ p \ as p in pepper	\ zh \ as si in vision

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Medical Dictionary

One entry found for **multifactorial**.

Main Entry: **mul·ti·fac·to·ri·al**
Pronunciation: -fak-ˈtōr-ē-əl, -ˈtȯr-
Function: *adjective*
1 : having characters or a mode of inheritance dependent on a number of genes at different loci
2 *or* **mul·ti·fac·tor** /-ˈfak-tər/ : having, involving, or produced by a variety of elements or causes <a *multifactorial* study> <a disease with a *multifactorial* etiology>
- **mul·ti·fac·to·ri·al·ly** /-ē-ə-lē/ *adverb*
- **mul·ti·fac·to·ri·al·i·ty** /-ˌtōr-ē-ˈal-ət-ē, -ˌtȯr-/ *noun, plural* **-ties**

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Pronunciation Key

\ ə \ as a in abut	\ g \ as g in go	\ r \ as r in red
\ ˈə ɪə \ as u in abut	\ h \ as h in hat	\ s \ as s in less>
\ ᵊ \ as e in kitten	\ i \ as i in hit	\ sh \ as sh in shy
\ ər \ as ur/er in further	\ ī \ as i in ice	\ t \ as t in tie
\ a \ as a in ash	\ j \ as j in job	\ th \ as th in thin
\ ā \ as a in ace	\ k \ as k in kin	\ th \ as th in the
\ ä \ as o in mop	\ k̲ \ as ch in ich dien	\ ü \ as oo in loot
\ aů \ as ou in out	\ l \ as l in lily	\ ů \ as oo in foot
\ b \ as in baby	\ m \ as m in murmur	\ v \ as v in vivid
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\ d \ as d in did	\ ŋ \ as ng in sing	\ y \ as y in yet
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\ ˈē ıē \ as ea in easy	\ ȯ \ as aw in law	\ yů \ as u in curable
\ ē \ as y in easy	\ ȯi \ as oy in boy	\ z \ as z in zone
\ f \ as f in fifty	\ p \ as p in pepper	\ zh \ as si in vision

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: James P. Elia	)	
	)	
SERIAL NO.: 09/794,456	)	EXAMINER: E.C. Kemmerer, Ph.D.
	)	
FILED: February 27, 2001	)	
	)	GROUP ART UNIT: 1646
FOR: METHOD FOR REPAIRING A DAMAGED PORTION OF A HUMAN HEART	)	

SECOND SUPPLEMENTAL DECLARATION
OF RICHARD HEUSER, M.D., F.A.C.C., F.A.C.P.

I, Richard Heuser, declare as follows:

1. I have offices at 500 West Thomas Road, Suite 900, Phoenix, Arizona 85013.

2. This Second Supplemental Declaration is submitted in addition to my previous Declaration, dated June 5, 2003 and my Supplemental Declaration dated February 4, 2004. No changes are made to either of such previous Declarations.

3. My Curriculum Vitae (hereinafter "CV") is attached as Exhibit A to my Declaration of June 5, 2003.

4. It is my understanding that the Examiner in charge of the above-identified patent application, in an Office Action dated June 1, 2004, questioned my qualification, for the first time, to render my previous opinions mentioned in above Paragraph 2. It is my further understanding that the basis for such questioning was that the Examiner noted that I did not report experience with cellular therapy. I desire to provide the information contained in following paragraph 5 to respond to the Examiner's newly raised question.

5. I am currently Director of Cardiovascular Research at St. Joseph's Hospital and Medicine Center, and I serve as Clinical Professor of Medicine at University of Arizona College of Medicine. Over the past six years, I have worked in gene therapy, as well as muscle regeneration for the treatment of cardiomyopathy.

In my CV, you will note reference to work that was done with Sulzer Medical involving a rabbit hind limb model to stimulate peripheral vascular disease. I injected a growth mixture that included FGF, etc. into the hind limb model.

In my U.S. Patent No. 6,190,379 entitled "Hot Tip Catheter," I developed a technique to deliver radiofrequency (PMR). In the full embodiment of the patent, I discuss delivery of protein and/or muscle cells in the myocardium using the inventive technique.

I have been involved as a member of the scientific advisory board with the world leader in cardiomyocyte regeneration, Bioheart, Miami Lakes, Florida. This company has been involved with laboratory and clinical trials using skeletal muscle cultured and modified. The sample is then delivered into the myocardium via a surgical or catheter approach.

6. I have read and understood the disclosures of the above-referenced patent application at page 20, line 10 through page 21, line 15; and page 44, line 19 through page 46, line 16. Such disclosures are the same as I read and understood in my previous Declaration and Supplemental Declaration. A copy of such disclosures is attached hereto as Second Supplemental Declaration Exhibit A.

7. I believe that one skilled in the medical arts, upon reading the disclosures in above Paragraph 6, would understand that cellular growth factors, such as multifactorial and non-specific cells, are included in such disclosures. Moreover, such skilled person would understand the disclosure on page 45 to be authored as an illustration of various modes of delivery of growth factors, whether they are genes or other genetic material; and that such

skilled person would further understand that the disclosures on pages 45 and 46 describe genetic material to include appropriate cells and genes.

8. Declarant states that the above opinion was reached independently.

Declarant understands that (1) any willful false statements and the like made herein are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon, and (2) that all statements made of Declarant's own knowledge are true and that all statements made on information and belief are believed to be true.

Further Declarant sayeth not.

Date: 7/18/04

Richard Heuser, M.D., F.A.C.C., F.A.C.P.

SECOND SUPPLEMENTAL DECLARATION

EXHIBIT A

DISCLOSURES

SECOND SUPPLEMENTAL DECLARATION
EXHIBIT A

DISCLOSURES
APPLICATION SERIAL NO. 09/794,456

PAGE 20, LINE 10 – PAGE 21, LINE 15

Growth factors can be utilized to induce the growth of "hard tissue" or bone and "soft tissues" like ectodermal and mesodermal tissues. As used herein, the term growth factor encompasses compositions and living organisms which promote the growth of hard tissue, such as bone, or soft tissue, in the body of a patient. The compositions include organic and inorganic matter. The compositions can be genetically produced or manipulated. The living organisms can be bacteria, viruses, or any other living organism which promote tissue growth. By way of example and not limitation, growth factors can include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (acidic/basis (FGF a,b), interleukins (IL's), tumor necrosis factor (TNF), transforming growth factor (TGF-B), colony-stimulating factor (CSF), osteopontin (Eta-1 OPN), platelet-derived growth factor (PDGF), interferon (INF), bone morphogenic protein 1 (BMP-1), and insulin growth factor (IGF). Recombinant and non-recombinant growth factors can be utilized as desired. Bacteria or viruses can, when appropriate, be utilized as growth factors. For example, there is a bacterial hydrophilic polypeptide that self-assembles into a nanometer internal diameter pore to build a selective lipid body. Various enzymes can be utilized for the synthesis of peptides which contain amino acids that control three-dimensional protein structure and growth. Growth factors can be applied in gels or other carriers which regulate the rate of release of the growth factors and help maintain the growth factors and the carrier, at a desired location in the body. Time release capsules, granules, or

other carriers containing growth factor can be activated by tissue pH, by enzymes, by ultrasound, by electricity, by heat, by selected *in vivo* chemicals or by any other selected means to release the growth factor. The carrier can be resorbable or non-resorbable. Or, the growth factor itself can be activated by similar means. Either the carrier or the growth factor can mimic extracellular fluid to control cell growth, migration, and function. The growth factor can be administered orally, systemically, in a carrier, by hypodermic needle, through the respiratory tract, or by any other desired method. The growth factor an also be administered into a capsule or other man-made composition or structure placed in the body. While administration of the growth factor is presently usually localized in the patient's body, circumstances may arise where it is advantageous to distribute a growth factor throughout the patient's body in uniform or non-uniform concentrations. An advantage to growth factors is that they can often, especially when in capsule form or in some other containment system, be inserted to a desired site in the body by simply making a small incision and inserting the growth factor. The making of such small incision comprises minor surgery which an often be accomplished on an out-patient basis. The growth factors can be multifactorial and nonspecific.

PAGE 44, LINE 19 – PAGE 46, LINE 16

Genetic material comprising a portion of a gene, a gene, genes, a gene product (i.e., a composition a gene causes to be produced like, for example, an organ-producing growth factor), growth factor, or an ECM (extracellular matrix) can be used in or on the body to grow an organ to tissue. For example, the vascular epithelial growth factor gene (VEGF) or its growth factor equivalent can be inserted into the body to cause an artery to grow. When insertion of a gene, portion of a gene, gene product, growth factor, or ECM *in vivo* or *ex vivo* is referred to herein in

connection with any of the implant techniques of the invention, it is understood that a cell nutrient culture(s), physiological nutrient culture(s), carrier (s), enhancer(s), promoter(s), or any other desired auxiliary component(s) can be inserted with the gene or at the same location as the gene, growth factor, ECM, etc.

An artery is an organ from the circulatory system. An artery can be grown in the heart, legs, or other areas by injecting a gene or other genetic material into muscle at a desired site. Size, vascularity, simplicity of access, ease of exploitation, and any other desired factors can be utilized in selecting a desired site. The gene is one of several known VEGF genes which cause the production of vascular endothelial growth factors. Several VEGF genes which produce vascular endothelial growth factors are believed to exist because nature intends for there to be several pathways (i.e., genes) which enable the production of necessary growth factors. The existence of several pathways is believed important because if one of the genes is damaged or inoperative, other similar genes can still orchestrate the production of necessary growth factors. VEGF genes are used by the body to promote blood vessel growth. VEGF genes are assimilated (taken in) by muscle cells. The genes cause the muscle cells to make a VEGF protein which promotes the growth of new arteries. VEGF proteins can be made in a lab and injected into a patient intravenously, intraluminally, or intramuscularly to promote the growth of an artery. Or, the genes (or other genetic material) can be applied with an angioplasty balloon, with the assistance of a vector, or by any other method.

It is not always desirable to grow a completely new organ. Sometimes growing a portion of an organ is desirable. For example, in some heart attacks or strokes, a portion of the heart or brain remains viable and a portion dies. An injection of a gene to form cardiac muscle and/or an injection of a gene to form an artery can be utilized to revive or replace the dead portion of the

heart. The dead portion of the heart may (or may not) be used as a matrix while the new muscles and vessels grow. Thus, in this example, a partial new organ is grown in a pre-existing organ. A pacemaker may (or may not) be necessary. A second injection of a gene may (or may not) be necessary to stop cardiac muscle growth once it is completed. Portions of organs throughout the body can similarly be repaired or replaced. It may be necessary to provide gene(s) or growth factor(s) sequentially. For instance, one or more blood vessels are grown by inserting an appropriate gene or other genetic material into a selected area. Second, an appropriate gene or other genetic material is inserted in the selected area to grow a bone or other organ.

The size and shape limitation of the desired structure can come from a containment and boundary contact inhibition phenomenon or by a chemical inhibition.

A variation on the theme of growing a portion of an organ is as follows: a portion of a heart dies. The pericardium is utilized as a scaffold and seeded with cells and/or genes to grow new muscle, and genes (or other genetic material) to grow new arteries. Immediately adjacent the dead cardiac muscle, onto or into the pericardium, the appropriate cells, genes, and/or growth factors (or other genetic material) are placed. Once the new muscle and blood vessels have grown, the function specific tissue can be applied to the damaged portion of the heart and paced, if necessary, to augment cardiac action. If the surgeon desires, the dead muscle can be removed and the new muscle and blood vessels can be surgically rotated into the excised region and secured. This probably can be done endoscopically. In essence, the pericardium is utilized to allow the new muscle wall to grow. The new muscle wall is then transplanted into the damaged heart wall. This procedure utilizes the body as a factor to grow an organ and/or tissue, after which the organ and/or tissue is transplanted to a desired region. On the other hand, the new muscle wall may integrate itself into the old wall and not require transplantation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: James P. Elia
SERIAL NO.: 09/794,456
FILED: February 27, 2001
FOR: METHOD FOR REPAIRING A DAMAGED PORTION OF A HUMAN HEART

EXAMINER: E.C. Kemmerer, Ph.D.

GROUP ART UNIT: 1646

SECOND SUPPLEMENTAL DECLARATION
OF ANDREW E. LORINCZ, M.D.

I, Andrew E. Lorincz, declare as follows:

1. I reside at 13820 NW County Rd 235, Apt 8, Alachua, FL 32616-2098.

2. This Second Supplemental Declaration is submitted in addition to my previous Declaration dated June 5, 2003 and my Supplemental Declaration dated February 3, 2004. No changes are made to either of such previous Declarations.

3. My Curriculum Vitae (hereinafter "CV") is attached as Exhibit A to my previous Declaration.

4. It is my understanding that the Examiner in charge of the above-identified patent application, in an Office Action dated June 1, 2004 for related patent application

Serial No. 09/794,456, questioned my qualification, for the first time, to render my previous opinions mentioned in above Paragraph 2. It is my further understanding that the basis for such questioning was that the Examiner noted that I did not report experience with cellular therapy. I desire to provide the information contained in following paragraph 5 so that the Examiner can consider such information in this application, as well.

5. In addition to the qualifications set forth in my CV, I am familiar with stem cell technology, including bone marrow preparation.

6. I have read and understood the disclosures of the above-referenced patent application at page 20, line 10 through page 21, line 15; and page 44, line 19 through page 46, line 16. Such disclosures are the same as I read and understood in my previous Declaration and Supplemental Declaration. A copy of such disclosures is attached hereto as Second Supplement Declaration Exhibit A.

7. I believe that one skilled in the medical arts, upon reading the disclosures in above Paragraph 6, would understand that cellular growth factors, such as multifactorial and non-specific cells, are included in such disclosures. Moreover, such skilled person would understand the disclosure on page 45 to be authored as an illustration of various modes of delivery of growth factors, whether they are genes or other genetic material; and that such skilled person would further understand that the disclosures on pages 45 and 46 describe genetic material to include appropriate cells and genes.

8. Declarant states that the above opinion was reached independently.

Declarant understands that (1) any willful false statements and the like made herein are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon, and (2) that all statements made of Declarant's own knowledge are true and that all statements made on information and belief are believed to be true.

Further Declarant sayeth not.

Date: 7-19-04

Andrew E. Lorincz, M.D.

SECOND SUPPLEMENTAL DECLARATION
EXHIBIT A

DISCLOSURES
APPLICATION SERIAL NO. 09/794,456

PAGE 20, LINE 10 – PAGE 21, LINE 15

Growth factors can be utilized to induce the growth of "hard tissue" or bone and "soft tissues" like ectodermal and mesodermal tissues. As used herein, the term growth factor encompasses compositions and living organisms which promote the growth of hard tissue, such as bone, or soft tissue, in the body of a patient. The compositions include organic and inorganic matter. The compositions can be genetically produced or manipulated. The living organisms can be bacteria, viruses, or any other living organism which promote tissue growth. By way of example and not limitation, growth factors can include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (acidic/basis (FGF a,b), interleukins (IL's), tumor necrosis factor (TNF), transforming growth factor (TGF-B), colony-stimulating factor (CSF), osteopontin (Eta-1 OPN), platelet-derived growth factor (PDGF), interferon (INF), bone morphogenic protein 1 (BMP-1), and insulin growth factor (IGF). Recombinant and non-recombinant growth factors can be utilized as desired. Bacteria or viruses can, when appropriate, be utilized as growth factors. For example, there is a bacterial hydrophilic polypeptide that self-assembles into a nanometer internal diameter pore to build a selective lipid body. Various enzymes can be utilized for the synthesis of peptides which contain amino acids that control three-dimensional protein structure and growth. Growth factors can be applied in gels or other carriers which regulate the rate of release of the growth factors and help maintain the growth factors and the carrier, at a desired location in the body. Time release capsules, granules, or

other carriers containing growth factor can be activated by tissue pH, by enzymes, by ultrasound, by electricity, by heat, by selected *in vivo* chemicals or by any other selected means to release the growth factor. The carrier can be resorbable or non-resorbable. Or, the growth factor itself can be activated by similar means. Either the carrier or the growth factor can mimic extracellular fluid to control cell growth, migration, and function. The growth factor can be administered orally, systemically, in a carrier, by hypodermic needle, through the respiratory tract, or by any other desired method. The growth factor an also be administered into a capsule or other man-made composition or structure placed in the body. While administration of the growth factor is presently usually localized in the patient's body, circumstances may arise where it is advantageous to distribute a growth factor throughout the patient's body in uniform or non-uniform concentrations. An advantage to growth factors is that they can often, especially when in capsule form or in some other containment system, be inserted to a desired site in the body by simply making a small incision and inserting the growth factor. The making of such small incision comprises minor surgery which an often be accomplished on an out-patient basis. The growth factors can be multifactorial and nonspecific.

PAGE 44, LINE 19 – PAGE 46, LINE 16

Genetic material comprising a portion of a gene, a gene, genes, a gene product (i.e., a composition a gene causes to be produced like, for example, an organ-producing growth factor), growth factor, or an ECM (extracellular matrix) can be used in or on the body to grow an organ to tissue. For example, the vascular epithelial growth factor gene (VEGF) or its growth factor equivalent can be inserted into the body to cause an artery to grow. When insertion of a gene, portion of a gene, gene product, growth factor, or ECM *in vivo* or *ex vivo* is referred to herein in

connection with any of the implant techniques of the invention, it is understood that a cell nutrient culture(s), physiological nutrient culture(s), carrier (s), enhancer(s), promoter(s), or any other desired auxiliary component(s) can be inserted with the gene or at the same location as the gene, growth factor, ECM, etc.

An artery is an organ from the circulatory system. An artery can be grown in the heart, legs, or other areas by injecting a gene or other genetic material into muscle at a desired site. Size, vascularity, simplicity of access, ease of exploitation, and any other desired factors can be utilized in selecting a desired site. The gene is one of several known VEGF genes which cause the production of vascular endothelial growth factors. Several VEGF genes which produce vascular endothelial growth factors are believed to exist because nature intends for there to be several pathways (i.e., genes) which enable the production of necessary growth factors. The existence of several pathways is believed important because if one of the genes is damaged or inoperative, other similar genes can still orchestrate the production of necessary growth factors. VEGF genes are used by the body to promote blood vessel growth. VEGF genes are assimilated (taken in) by muscle cells. The genes cause the muscle cells to make a VEGF protein which promotes the growth of new arteries. VEGF proteins can be made in a lab and injected into a patient intravenously, intraluminally, or intramuscularly to promote the growth of an artery. Or, the genes (or other genetic material) can be applied with an angioplasty balloon, with the assistance of a vector, or by any other method.

It is not always desirable to grow a completely new organ. Sometimes growing a portion of an organ is desirable. For example, in some heart attacks or strokes, a portion of the heart or brain remains viable and a portion dies. An injection of a gene to form cardiac muscle and/or an injection of a gene to form an artery can be utilized to revive or replace the dead portion of the

heart. The dead portion of the heart may (or may not) be used as a matrix while the new muscles and vessels grow. Thus, in this example, a partial new organ is grown in a pre-existing organ. A pacemaker may (or may not) be necessary. A second injection of a gene may (or may not) be necessary to stop cardiac muscle growth once it is completed. Portions of organs throughout the body can similarly be repaired or replaced. It may be necessary to provide gene(s) or growth factor(s) sequentially. For instance, one or more blood vessels are grown by inserting an appropriate gene or other genetic material into a selected area. Second, an appropriate gene or other genetic material is inserted in the selected area to grow a bone or other organ.

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FOCUS ISSUE: CARDIAC REGENERATION

Regeneration of Human Infarcted Heart Muscle by Intracoronary Autologous Bone Marrow Cell Transplantation in Chronic Coronary Artery Disease

The IACT Study

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Düsseldorf, Germany

OBJECTIVES Stem cell therapy may be useful in chronic myocardial infarction (MI); this is conceivable, but not yet demonstrated in humans.

BACKGROUND After acute MI, bone marrow-derived cells improve cardiac function.

METHODS We treated 18 consecutive patients with chronic MI (5 months to 8.5 years old) by the intracoronary transplantation of autologous bone marrow mononuclear cells and compared them with a representative control group without cell therapy.

RESULTS After three months, in the transplantation group, infarct size was reduced by 30% and global left ventricular ejection fraction (+15%) and infarction wall movement velocity (+57%) increased significantly, whereas in the control group no significant changes were observed in infarct size, left ventricular ejection fraction, or wall movement velocity of infarcted area. Percutaneous transluminal coronary angioplasty alone had no effect on left ventricular function. After bone marrow cell transplantation, there was an improvement of maximum oxygen uptake (Vo_{2max}, +11%) and of regional ^{18}F-fluor-desoxy-glucose uptake into infarct tissue (+15%).

CONCLUSIONS These results demonstrate that functional and metabolic regeneration of infarcted and chronically avital tissue can be realized in humans by bone marrow mononuclear cell transplantation. (J Am Coll Cardiol 2005;46:1651–8) © 2005 by the American College of Cardiology Foundation

Cardiac performance after myocardial infarction (MI) is compromised by ventricular remodeling, which represents a major cause of late infarct-related chronic heart failure and death (1,2). Although conventional drug therapy (e.g., with beta-receptor blockers and/or angiotensin-converting enzyme inhibitors) may delay remodeling, there is no basic

See page 1659

therapeutic regimen available for preventing or even reversing this process. By the use of interventional therapeutics (percutaneous transluminal coronary angioplasty [PTCA], stent), recanalization of the occluded infarct-related artery is possible, thereby improving or normalizing coronary blood flow. However, despite sufficient reperfusion of infarcted tissue, the viability of the infarcted myocardium cannot, or can only insufficiently, be improved in most of these patients (3). Therefore, catheter-based therapy of acute MI is useful for vascular recanalization, but the second and crucial step, the regeneration of necrotic heart muscle, is not realized by this vascular procedure alone.

Experimental (4) and clinical (5,6) studies have shown recently for the first time that bone marrow mononuclear cells (BMCs) may regenerate damaged myocardium in acute MI in humans. Because the regenerative potential of bone marrow-derived cells ought also to be expected to exist in chronically ischemic heart disease as well (7–12), we have assembled in an ongoing clinical investigation 18 patients with chronic MI to prove this new therapeutic possibility.

METHODS

Study population. All 18 patients (49 ± 11 years) were men and were recruited consecutively from January 2003 until March 2004. They had had transmural MI 27 ± 31 months before, at which point all infarcts had been treated acutely by PTCA and/or stent implantation (Table 1, Fig. 1).

The inclusion criteria were age <70 years, one-vessel disease with an open infarct-related artery at the time of stem cell therapy, sinus rhythm, a clear-cut demarcation of the ventriculographic infarct area, and no coronary bypass surgery. General exclusion criteria were severe comorbidity and alcohol or drug dependency. Although chronically infarcted myocardium usually does not regenerate sponta-

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November 1, 2005:1651–8

Abbreviations and Acronyms

BMC = bone marrow mononuclear cell
CPK = creatine phosphokinase
ECG = electrocardiogram
LV = left ventricular
MI = myocardial infarction
PET = positron emission tomography
PTCA = percutaneous transluminal coronary angioplasty
Tx group = transplantation group

neously, for comparison a control group, parallel to the recruitment of the stem cell transplantation group (Tx group), was recruited and analyzed, meeting the same inclusion criteria as the stem-cell group. The recruitment of patients was performed according to a randomization procedure in which all patients of the entire chronic infarction group were distributed to the treatment group, where they agreed with all the therapeutic regimen. Alternatively, all patients of the chronic infarction group who refused the therapeutic regimen (bone marrow puncture and aspiration, intracoronary cell transplantation, and another cardiac catheterization) were allocated to the control group. All medications with angiotensin-converting enzyme inhibitors and with beta receptor blockers were maintained constant during the study period.

The cell-treated patients had stable ventricular dynamics for infarct size, ejection fraction, and wall movement velocity of infarcted area at least 9 ± 6 months before cell transplantation. Infarct size at the time of cell therapy showed an amount of 27 ± 8% of the circumference of the left ventricle (LV), determined by ventriculography.

Preparation of BMCs. One day before cell therapy, bone marrow was taken (80 ml from the iliac crest) and mononuclear cells were isolated and identified including CD34-positive cells, AC133-positive cells and CD45/CD14 negative cells (6). The cells were isolated under good manufacturing practice conditions by Ficoll density separation on Lymphocyte Separation Medium (Bio Whittaker, Walkersville, Maryland), before the residual erythrocytes were lysed with H_2O. For overnight cultivation, 1×10^6 BMCs/ml were placed in Teflon bags (Vuelife, Cell Genix, Gaithersburg, Maryland) and cultivated in X-Vivo 15 Medium (Bio Whittaker) supplemented with 2% heat-inactivated autologous plasma. The next day, BMCs were harvested and washed three times with heparinized saline before final resuspension in heparinized saline. Viability was 93 ± 3%. Heparinization and filtration (cell strainer, FALCON) was carried out to prevent cell clotting and microembolization during intracoronary transplantation. These cells were used for therapy. All microbiologic tests of the clinically used cell preparations proved negative. All patients received extensive information about the procedure, which was approved by the ethical committee of our university, and all gave written informed consent.

Administration of BMCs. Following assessment of baseline examinations (coronary angiography, left ventriculography, spiroergometry, ^{99m}Tc-tetrofosmin single-photon emission computed tomography (SPECT) and ^{18}F-fluor-deoxy-glucose (^{18}F-FDG) positron emission tomography (PET), cell transplantation was performed via the intracoronary administration route (6,13) using four to six fractional infusions parallel to balloon inflation over 2 to 4 min of 3 to 5 ml of cell suspension, each containing 15 to 22 × 10^6 mononuclear cells. All cells were infused directly into the infarcted zone through the infarct-related artery via an angioplasty balloon catheter, which was inflated at a low pressure (2 to 4 atm) and was located within

Table 1. Demographic Data of Intracoronary Bone Marrow Stem Cell Transplantation Group and Control Group

Characteristics	Tx Group	Control Group	p
No. of patients	18	18	
Age, yrs	49 ± 11	52 ± 10	NS
Transmural myocardial infarction, months before Tx	27 ± 31	30 ± 34	NS
Coronary angiography			
LAD/LCX/RCA as affected vessel	16/0/2	10/3/5	
No. of patients with stent implantation	16	17	NS
Risk factors			
Diabetes mellitus, %	16	11	NS
Positive family history, %	44	33	NS
Smoker and ex-smoker, %	67	56	NS
Hyperlipoproteinemia, %	89	94	NS
Medication			
Beta-blocker, %	94	89	NS
Angiotensin-converting enzyme inhibitor, %	94	89	NS
Statin, %	94	100	NS
Laboratory parameters			
CPK, U/l	1,504 ± 979	1,489 ± 952	NS
Bone marrow mononuclear cells, n (10^6×)	90		

Values are mean ± SD or number of patients.
CPK = creatine phosphokinase; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RCA = right coronary artery; Tx = intracoronary bone marrow stem cell transplantation.

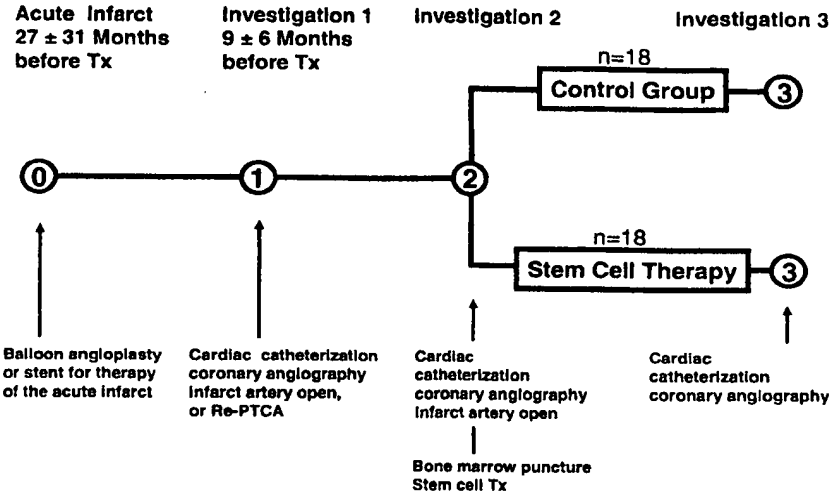

Figure 1. Diagrammatic representation of the algorithm of intracoronary stem cell therapy (Tx) in chronic ischemic heart disease after myocardial infarction. The infarcts occurred 27 ± 31 months before Tx. All infarct patients were treated with percutaneous transluminal coronary angioplasty (PTCA) or with stent implantation. 9 ± 6 months before (investigation 1) coronary angiography (including quantitative left ventriuclography) was performed. If re-stenosis was present, re-PTCA was made. Investigation 2 embraces all patients for the evaluation of coronary morphology after PTCA/stent. Only patients with an open infarct-related artery were included in both groups. Patients who agreed to Tx received within 10 days after investigation 2 bone marrow punctures and Tx by the intracoronary administration route and had altogether five invasive investigations, including two for therapeutic reasons (nos. 0 and 1). Patients who were not eligible for Tx (disagreement with bone marrow puncture and with subsequent Tx) served as a control group. Investigation 3 represents all follow-up measurements 3 months after Tx (Tx patients) or after investigation 2 for control group patients.

the previously stented coronary segments. This prevented backflow of cells and produced stop flow beyond the site of balloon inflation to facilitate high-pressure infiltration of cells into the infarcted zone. Prolonged contact time for cellular migration was also enabled. Three months after catheter-guided cell transplantation, all functional tests were repeated, including coronary angiography and left ventriculography. There were no procedural or cell-induced complications, and there were no side effects in any patient.

Spiroergometry. Aerobic exercise capacity was examined before (<10 days) intracoronary cell transplantation and three months later during follow-up. All patients (n = 18) were subjected to initial bicycle spiroergometry to assess their functional fitness and to determine the limit of safe intensity of exercise. We chose a protocol with an intensified workload up to the symptom-limited maximum (basic load of 50 W, intensification at 25 W, 2-min duration of each workload step). We determined the anaerobic threshold for prescribing a suitable load intensity. During the whole spiroergometry, monitoring by a 12-lead electrocardiogram (ECG) was carried out. The exercise capacity was assessed on the basis of maximum load levels expressed in watts (W_{max}) and maximum peak oxygen uptake (Vo_{2max}).

Coronary angiography and left ventriculography. Coronary angiography and biplane left ventriculography were performed 9 ± 6 months before cell transplantation and also a second time, within 10 days, immediately before cell therapy. The therapeutic follow-up was three months after the treatment. Thus, stable baseline conditions were documented (coronary vessel involvement, ventricular function, and geometry). Cardiac function was evaluated by left ventricular (LV) ejection fraction and by auxotonic myocardial contractility index, evaluated by the wall movement velocity of the infarcted area. The infarct size was calculated according to the method of Sheehan (14) by plotting five axes perpendicular to the long axis of the heart in the main akinetic or dyskinetic segment of the ventricular wall. Systolic and diastolic lengths were then measured by two independent observers, and the mean difference was divided by the systolic duration in seconds.

Quantification of coronary stenosis (restenosis). Cinecoronarangiograms were obtained during stem cell transplantation and at three months thereafter according to standard acquisition guidelines. The angiograms were evaluated by two independent observers and quantitative analysis was performed (15). Standard morphologic criteria were used to characterize the complexity of baseline lesions. The user-defined reference diameter proximal to the stenosis and the minimal luminal diameter within the culprit of the stenosis were used to calculate the percentage of stenosis. A value of 0 mm was assigned for the minimal luminal diameter in case of total occlusion at baseline or follow-up. Restenosis was defined as ≥50% stenosis of the initial target lesion at follow-up. Calculations of restenosis were performed in both groups, with and without stem cell therapy, in the same way, thus enabling evaluation the differential effects of PTCA-guided cell therapy and of PTCA effects alone.

Ventricular function after PTCA in the control group. For the evaluation of a potential effect on the PTCA intervention itself on LV function, all patients in the control group were analyzed with regard to infarct size, ejection fraction, and infarction wall movement velocity.

Table 2. Single Values of Intracoronary Bone Marrow Stem Cell Transplantation Group

Patient Number	Area of Infarction, %*			LV Ejection Fraction, %*			Infarction Wall Movement Velocity, cm/s*		
	Investigation 1 9 ± 6 Mo Before Tx	Investigation 2 <10 Days Before Tx	Investigation 3 3 Mo After Tx	Investigation 1 9 ± 6 Mo Before Tx	Investigation 2 <10 Days Before Tx	Investigation 3 3 Mo After Tx	Investigation 1 9 ± 6 Mo Before Tx	Investigation 2 <10 Days Before Tx	Investigation 3 3 Mo After Tx
1	26	26	22	56	55	60	0.88	0.77	0.82
2	28	29	26	45	43	49	2.06	1.88	2.13
3	16	16	5	64	65	71	1.45	1.50	2.10
4	27	25	14	48	50	65	1.20	1.25	2.88
5	16	14	11	66	69	71	2.25	2.77	3.75
6	16	13	6	64	66	73	1.50	1.77	2.55
7	15	18	11	57	55	63	2.78	2.65	3.13
8	28	28	20	43	44	49	3.15	3.25	4.25
9	27	27	11	46	46	64	1.61	1.65	3.30
10	20	17	14	56	58	62	2.21	2.45	3.13
11	28	25	17	42	38	52	1.91	1.88	3.00
12	33	28	21	44	47	54	2.28	2.62	3.50
13	39	37	27	50	51	59	1.25	2.50	4.90
14	29	33	27	62	62	61	1.20	1.33	2.70
15	37	37	31	48	43	53	1.83	1.56	2.50
16	29	29	24	53	54	58	1.25	1.06	3.06
17		41	35		48	55		1.66	3.00
18		35	25		45	53		0.94	1.94
Mean	26	27	19	53	52	60	1.80	1.86	2.92
SD	7	8	9	8	9	7	0.63	0.70	0.91

*Calculated from left ventriculography.
LV = left ventricular; Mo = Months; other abbreviations as in Table 1.

Nuclear cardiologic investigations (PET and SPECT). ^{18}F-FDG-positron emission tomography (^{18}F-FDG PET) was performed with a Scanditronix SCX 4096 WB-Scanner (FWHM = 6 mm transaxial, axial field of view = 4.6 cm). Patients received an oral glucose load of 1 g/kg body weight 80 ± 30 min before the intravenous application of ^{18}F-FDG (380 ± 60 MBq). The ^{18}F-FDG was administered at the time of decrease of blood glucose level <130 mg/dl. An initial transmission scan was obtained using a ^{68}Ga-filled pin source to correct the subsequent emission scans for attenuation. The data acquisition was started 45 min after administration of FDG. Image data were recorded with a 256 × 256 matrix in 3 consecutive bed positions over 15 min per position. The data were reconstructed back-projected with a Hanning filter (5 mm).

^{99m}Tc-tetrofosmin SPECT. Sixty minutes after intravenous injection of 600 ± 140 MBq of the perfusion-marker ^{99m}Tc-tetrofosmin under a "rest" condition, the images were obtained using a SPECT scanner with double-head detector (PRISM 2000, Marconi/Phillips), a low-energy, high-resolution collimator, and a 128 × 128 matrix. Image data were collected over 360° at 3° every 30 s. The images were reconstructed backprojected with a low-pass filter (order 12, cutoff 0.2).

PET and SPECT evaluation. Normalized values for FDG uptake and perfusion were calculated by comparing regional with maximum tracer uptake on the reconstructed images. We performed a regional analysis of glucose metabolism and perfusion using a set of standardized, individually adjusted circular regions of interest (diameter 18.06 mm, surface 256 mm^2). The reconstructed metabolic and perfusion images were realigned for each patient (MPI-Tool, version 3.0; Advanced Tomo Vision, Erftstadt, Germany) and were resliced according to cardiac axis (short-axis and horizontal and vertical long-axis views). The regions were positioned immediately neighboring, with no overlap, according to an overlay of the co-registrated metabolic and perfusion images. The regions covered the infarct lesion as well as normal myocardium. In this way, we generated templates of regions for each patient, which could be used for the evaluation of metabolism and perfusion, before and after BMC transplantation without further modification. According to Segall et al. (16), regions with a normalized FDG uptake <50% were rated as transmural scar and regions with an uptake of 50% to 60% as non-transmural scar.

Further analysis was restricted to regions with FDG uptake <60% in the PET scans, pursuant to our intention to focus on the effects of BMC transplantation on scar tissue.

Safety parameters. To assess any inflammatory response and myocardial reaction after cell therapy, white blood cell count, the serum levels of C-reactive protein (CRP) and of creatine phosphokinase (CPK) were determined immediately before as well as after treatment. Additional analysis was done directly after transplantation and three months later: ECG at rest, 24-h Holter ECG, and echocardiography.

Statistical analysis. All data are presented as mean ± SD. Statistical significance was accepted when $p < 0.05$. Intra-individual comparison of variables of investigation 1 (9 ± 6 months before cell transplantation for Tx group, 9 ± 5 months before investigation 2 for control patients) and investigation 2 (<10 days before cell transplantation for Tx group, no transplantation for control patients) and of variables of investigation 2 and follow-up investigation 3 (3 months after cell therapy for Tx group, 8 ± 5 months after investigation 2 for control patients) was performed using Wilcoxon rank-sum test. The missing values (Table 2) were omitted and not calculated for statistical analysis. The p values (by analysis of variance) have been given for LV ejection fraction, area of infarction, and infarction wall movement velocity. Statistical analysis was performed with SPSS-Windows 10.1 software.

RESULTS

Three months after intracoronary cell therapy, the infarct size was reduced by 30%, whereas the global LV ejection fraction increased by 15% and regional infarct wall movement velocity by 57% (Tables 2 and 3). In parallel, the clinical performance improved (Table 4), as evidenced by a higher work load demonstrated by a 11% increase in maximum oxygen uptake (Vo_{2max}). SPECT investigation presented enhanced tetrofosmin uptake in the infarcted zone by 5%, and PET examination showed enhanced glucose uptake in the infarcted zone by 15%, demonstrating regeneration of formerly avital, chronically infarcted heart muscle (Fig. 2). An unchanged or even impaired LV function was not observed in any patient.

In the control group (18 patients with chronic MI, but without stem cell therapy) no significant changes were observed in infarct size, LV ejection fraction, or wall

Table 3. Cardiac Parameters in the Transplantation Group and in Control Group at the Three Investigation Time Points

	Area of Infarction, %			LV Ejection Fraction, %			Infarction Wall Movement Velocity, cm/s		
	Control Group	Tx Group	p Value*	Control Group	Tx Group	p Value*	Control Group	Tx Group	p Value*
Investigation 1	25 ± 9	26 ± 7	0.99	53 ± 10	53 ± 8	0.87	1.95 ± 0.66	1.80 ± 0.63	0.57
Investigation 2	27 ± 9	27 ± 8	0.83	51 ± 10	52 ± 9	1.00	1.88 ± 0.76	1.86 ± 0.70	0.94
Investigation 3	26 ± 9	19 ± 9	0.02	52 ± 10	60 ± 7	0.02	1.91 ± 0.79	2.92 ± 0.91	0.001

*Analysis of variance.
Abbreviations as in Table 1.

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November 1, 2005:1651–8

Table 4. Positron Emission Tomography and Spiroergometry Before and After Stem Cell Therapy in Chronically Infarcted Myocardium

	^{18}F-FDG-Positron Emission Tomography			VO_{2max}, Spiroergometry		
	FDG Uptake, %		Difference in %	ml/min		Difference in %
Investigation 1	none			none		
Investigation 2	43.8 ± 8.0	>	+ 15	1,602 ± 533	>	+ 11
Investigation 3	50.5 ± 11.6			1,776 ± 523		
p (Wilcoxon test)	0.012			0.0001		

^{18}F-FDG = ^{18}F-fluor-deoxy-glucose; VO_{2max} = maximum oxygen uptake.

movement velocity of the infarcted area (Figs. 3A to 3C). Electrocardiogram at rest and on exercise and 24-h Holter ECG revealed no rhythm disturbances at any time point. Only 1 patient (from 18 cell-treated patients, 6%) developed relevant restenosis due to quantitative angiographic criteria. The restenosis could be treated adequately by stent implantation. The other 17 patients showed good patency rates without restenosis after PCI and cell transplantation. They also revealed no alterations in LV function 8 ± 5 months after PTCA.

There was no inflammatory response or myocardial reaction (white blood cell count, CRP, CPK) after cell therapy, despite a moderate increase in CRP (before cell transplantation 0.58 ± 0.48 mg/dl, after cell transplantation 1.07 ± 0.73 U/l, p = 0.002), which is usual after bone marrow puncture and/or cardiac catheterization.

DISCUSSION

The results of these investigations demonstrate, for the first time, that the intracoronary transplantation of autologous bone marrow mononuclear cells may reduce infarct size and improve LV function as well as myocardial glucose uptake in chronic ischemic heart disease attributable to chronic MI (5 months to 8.5 years old). Infarct size decreased in all patients and cardiac performance (ejection fraction, wall movement velocity of infarcted area, maximum oxygen uptake, and exercise tolerance) and myocardial metabolism (FDG-PET) improved, all being between 11% and 57%. Furthermore, it is noteworthy that there were no complications immediately or three months after cell transplantation, especially that there was no cardiac arrhythmia and no signs of cardiac or systemic inflammation were present.

The effects of stem cell transplantation on infarct size, cardiac function, and contractility demonstrate significant improvement of these three parameters in the therapy group (before and after stem cell therapy) as well as in the comparison between the stem cell therapy group and the control group, thus giving evidence for a beneficial therapeutic effect of stem cell therapy on cardiac performance in chronic MI.

Patients in both the stem-cell group and the control group were recruited in parallel to each other and consecutively between January 2003 and March 2004. They all (n = 36) fulfilled the same inclusion criteria. Thus, representative patient characteristics were present for the stem cell group (n = 18) and the control group (n = 18) as well as in comparing both of them. Moreover, two subsequent investigations before stem cell transplantation have been performed for each patient: investigation 1 and 2 demonstrated the stability of LV dynamics before cell therapy (9 months respectively 10 days before transplantation) and investigation 3 compared the effects of stem cell therapy with the control group. The stable hemodynamics during the preceding 9 ± 6 months before stem-cell therapy and the stable hemodynamics within the control group at all three points of investigation underline the significant alterations of the left ventriculography-derived parameters investigated after stem cell transpantation.

The regenerative potential of bone-marrow–derived stem cells may be explained by any of four mechanisms: 1) direct cell differentiation from mononuclear cells to cardiac myocytes (17), 2) cytokine-induced growing and increase of residual viable myocytes, especially within the border zone of the infarcted area (18), 3) stimulation of intrinsic myocardial stem cells (endogenous stem cells) (19,20), and 4)

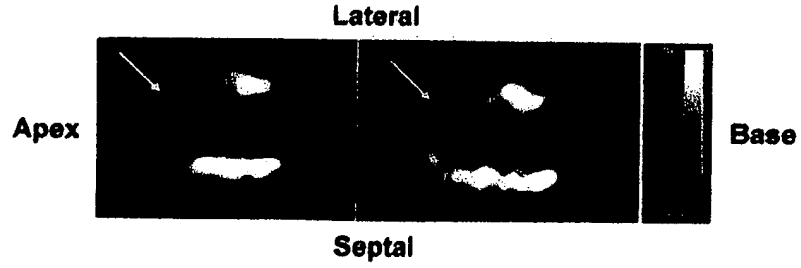

Figure 2. Representative illustration of ^{18}F-FDG-positron emission tomography (PET) before **(above)** and 3 months after **(below)** cell therapy in the transversal **(left)** and longitudinal **(right projection)** in a 30-year-old male patient with an 8-month-old anteroapical infarction. Note the restoration of glucose uptake **(below)** within the infarcted area of the formerly completely avital anteroapical myocardium.

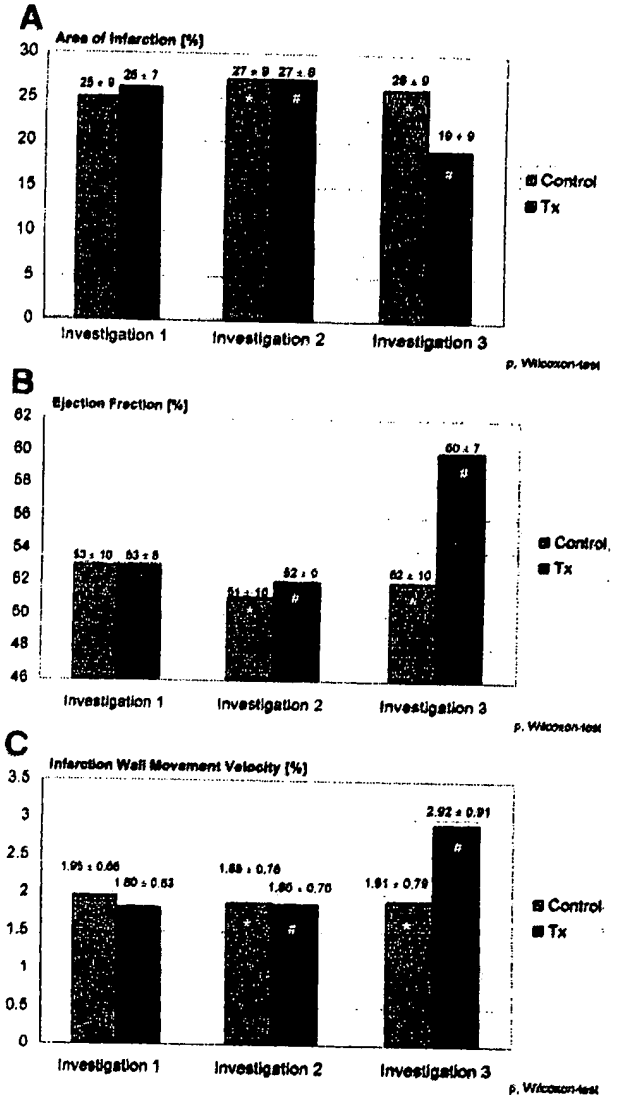

Figure 3. Illustration of the mean values of **(A)** area of infarction, **(B)** ejection fraction, and **(C)** infarction wall movement velocity, determined by quantitative left ventriculography in both groups (control group vs. transplantation [Tx] group) at the point of time: investigations 1, 2, and 3. Comparision of both groups with chronically infarcted myocardium (conrol group vs. Tx group), n = 18 patients. Investigation 1 was 9 ± 6 months before cell transplantation (controls: 9 ± 5 months before percutaneous transluminal coronary angioplasty [PTCA]); investigation 2 within 10 days before cell tranplantation (controls: at the time point of PTCA) and investigation 3 was three months after cell transplantation (controls: 8 ± 5 months after PTCA). Note the significant decrease of infarct size and the increase in ejection fraction and in contractility (infarction wall movement velocity) 3 months after cell therapy in comparison with the control group. *p = not significant (investigation 2 vs. investigation 3); #p = 0.001 (investigation 2 vs. investigation 3).

induction of cell fusion between transplanted bone marrow cells and resident myocytes (21–24).

Transdifferentiation has been described by previous investigators (4); however, it has been questioned by recent experimental studies (25). The influence of cytokines has shown to restore coronary blood vessels and muscle cells after experimental myocardial infarction. This regeneration of blood vessels and muscle cells is most pronounced in the border zone of ischemic and/or infarcted tissue (26), demonstrating an enhancement of mitotic cells and cell cycles up four-fold, when compared to areas remote from the necrotic myocardium. Moreover, mononuclear bone marrow stem cells contain a lot of cytokines (VEGF, insulin-like growth factor, platelet-derived growth factor, and so on), thereby stimulating residual normal myocytes for regeneration and proliferation and instrinsic myocardial stem cells (endogenous stem cells) for cell regeneration and for cell fusion (27–31).

Mitotic indexes are three to four times more frequent within the border zone of myocardial necrosis when compared with non-injured heart muscle (26). Moreover, 20% to 40% of intracoronarily transplanted bone-marrow–derived stem cells may be accumulated within the border zone of MI. There were no signs of apparent microcirculation disturbances because all patients had Thrombolysis In Myocardial Infarction flow grade 3. Thus, it is conceivable that in MI the border zone represents the optimum "niche" for exogenously transplanted stem cells, stimulating mitosis rates and heart muscle regeneration, preferably originating in and expanding from these areas. Cell fusion may also contribute to heart muscle regeneration, which takes its origin from the border zone, expanding gradually to the necrotic core of the infarcted area.

Our study cannot determine which cell-biologic and molecular mechanims are responsible for heart muscle repair or which of the studied factors may play the predominant role. However, the final functional outcome of this cell therapy demonstrates three main target effects: improvement in muscle function (pumping ability and contractility), myocardial perfusion (SPECT), and myocardial glucose metabolism (PET), thus giving evidence that heart muscle repair must have taken place by this intracoronary bone marrow cell transplantation procedure.

The clinical significance of this novel therapeutic approach may embrace a large number of patients with chronic coronary artery disease, preferably after previous or longstanding MI. It is conceivable that remodeling after infarction may be ameliorated or even stopped by this procedure. Thus, cell therapy may represent a new option of basic and causal therapy in chronic infarcted myocardium. It is an open question whether variations of the amount and kind of bone marrow cells, the administration technique, and the transplantation procedure itself, by enhanced environment and improvement of the angiogenic micromilieu, can further improve the milieu-dependent differentiation or regeneration of bone marrow cells in chronic infarcted heart disease. Therefore, our clinical results represent a stable basis to proceed to the next necessary step: to a larger prospective randomized study.

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Mesenchymal stem cells: building blocks for molecular medicine in the 21st century.

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Mesenchymal stem sells (MSCs) are present in a variety of tissues during human development, and in adults they are prevalent in bone marrow. From that readily available source, MSCs can be isolated, expanded in culture, and stimulated to differentiate into bone, cartilage, muscle, marrow stroma, tendon, fat and a variety of other connective tissues. Because large numbers of MSCs can be generated in culture, tissue-engineered constructs principally composed of these cells could be re-introduced into the in vivo setting. This approach is now being explored to regenerate tissues that the body cannot naturally repair or regenerate when challenged. Moreover, MSCs can be transduced with retroviral and other vectors and are, thus, potential candidates to deliver somatic gene therapies for local or systemic pathologies. Untapped applications include both diagnostic and prognostic uses of MSCs and their descendents in healthcare management. Finally, by understanding the complex, multistep and multifactorial differentiation pathway from MSC to functional tissues, it might be possible to manipulate MSCs directly in vivo to cue the formation of elaborate, composite tissues in situ.

Publication Types:

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NATIONAL INSTITUTES OF HEALTH
May 2000

Office of the Director

Stem Cells: A Primer

This primer presents background information on stem cells. It includes an explanation of what stem cells are; what pluripotent stem cells are; how pluripotent stem cells are derived; why pluripotent stem cells are important to science; why they hold such great promise for advances in health care; and what adult stem cells are.

Recent published reports on the isolation and successful culturing of the first human pluripotent stem cell lines have generated great excitement and have brought biomedical research to the edge of a new frontier. The development of these human pluripotent stem cell lines deserves close scientific examination, evaluation of the promise for new therapies, and prevention strategies, and open discussion of the ethical issues.

In order to understand the importance of this discovery as well as the related scientific, medical, and ethical issues, it is absolutely essential to first clarify the terms and definitions.

Definitions

DNA - abbreviation for deoxyribonucleic acid which makes up genes.

Gene - a functional unit of heredity which is a segment of DNA located in a specific site on a chromosome. A gene directs the formation of an enzyme or other protein.

Somatic cell - cell of the body other than egg or sperm.

Somatic cell nuclear transfer - the transfer of a cell nucleus from a somatic cell into an egg from which the nucleus has been removed.

Stem cells - cells that have the ability to divide for indefinite periods in culture and to give rise to specialized cells.

Pluripotent -capable of giving rise to most tissues of an organism.

Totipotent - having unlimited capability. Totipotent cells have the capacity to specialize into extraembryonic membranes and tissues, the embryo, and all postembryonic tissues and organs.

What is a stem cell?

Stem cells have the ability to divide for indefinite periods in culture and to give rise to specialized cells. They are best described in the context of normal human development. Human development begins when a sperm fertilizes an egg and creates a single cell that has the potential to form an entire organism. This fertilized egg is **totipotent**, meaning that its potential is total. In the first hours after fertilization, this cell divides into identical totipotent cells. (Figure I) This means that either one of these cells, if placed into a woman's uterus, has the potential to develop into a fetus. In fact, identical twins develop when two totipotent cells separate and develop into two individual, genetically identical human beings. Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize, forming a hollow sphere of cells, called a blastocyst. The blastocyst has an outer layer of cells and inside the hollow sphere, there is a cluster of cells called the inner cell mass.

The outer layer of cells will go on to form the placenta and other supporting tissues needed for fetal development in the uterus. The inner cell mass cells will go on to form virtually all of the tissues of the human body. Although the inner cell mass cells can form virtually every type of cell

found in the human body, they cannot form an organism because they are unable to give rise to the placenta and supporting tissues necessary for development in the human uterus. These inner cell mass cells are **pluripotent** — they can give rise to many types of cells but not all types of cells necessary for fetal development. Because their potential is not total, they are not totipotent and they are not embryos. In fact, if an inner cell mass cell were placed into a woman's uterus, it would not develop into a fetus.

The pluripotent stem cells undergo further specialization into stem cells that are committed to give rise to cells that have a particular function. Examples of this include blood stem cells which give rise to red blood cells, white blood cells and platelets; and skin stem cells that give rise to the various types of skin cells. These more specialized stem cells are called **multipotent**. (Figure II)

While stem cells are extraordinarily important in early human development, multipotent stem cells are also found in children and adults. For example, consider one of the best understood stem cells, the blood stem cell. Blood stem cells reside in the bone marrow of every child and adult, and in fact, they can be found in very small numbers circulating in the blood stream. Blood stem cells perform the critical role of continually replenishing our supply of blood cells — red blood cells, white blood cells, and platelets — throughout life. A person cannot survive without blood stem cells.

How are pluripotent stem cells derived?

At present, human pluripotent cell lines have been developed from two sources[1] with methods previously developed in work with animal models.

(1) In the work done by Dr. Thomson, pluripotent stem cells were isolated directly from the inner cell mass of human embryos at the blastocyst stage. Dr. Thomson received embryos from IVF (In Vitro Fertilization) clinics-these embryos were in excess of the clinical need for infertility treatment. The embryos were made for purposes of reproduction, not research. Informed consent was obtained from the donor couples. Dr. Thomson isolated the inner cell mass (see Figure III) and cultured these cells producing a pluripotent stem cell line.

(2) In contrast, Dr. Gearhart isolated pluripotent stem cells from fetal tissue obtained from terminated pregnancies. Informed consent was obtained from the donors after they had independently made the decision to terminate their pregnancy. Dr. Gearhart took cells from the region of the fetus that was destined to develop into the testes or the ovaries. Although the cells developed in Dr. Gearhart's lab and Dr. Thomson's lab were derived from different sources, they appear to be very similar. (Figure III)

The use of somatic cell nuclear transfer (SCNT) may be another way that pluripotent stem cells could be isolated. In studies with animals using SCNT, researchers take a normal animal egg cell and remove the nucleus (cell structure containing the chromosomes). The material left behind in the egg cell contains nutrients and other energy-producing materials that are essential for embryo development. Then, using carefully worked out laboratory conditions, a somatic cell - any cell other than an egg or a sperm cell - is placed next to the egg from which the nucleus had been removed, and the two are fused. The resulting fused cell, and its immediate descendants, are believed to have the full potential to develop into an entire animal, and hence are totipotent. As described in Figure I, these totipotent cells will soon form a blastocyst. Cells from the inner cell mass of this blastocyst could, in theory, be used to develop pluripotent stem cell lines. Indeed, any method by which a human blastocyst is formed could potentially serve as a source of human

pluripotent stem cells (Figure IV).

Potential Applications of Pluripotent Stem Cells

There are several important reasons why the isolation of human pluripotent stem cells is important to science and to advances in health care (Figure V). At the most fundamental level, pluripotent stem cells could help us to understand the complex events that occur during human development. A primary goal of this work would be the identification of the factors involved in the cellular decision-making process that results in cell specialization. We know that turning genes on and off is central to this process, but we do not know much about these "decision-making" genes or what turns them on or off. Some of our most serious medical conditions, such as cancer and birth defects, are due to abnormal cell specialization and cell division. A better understanding of normal cell processes will allow us to further delineate the fundamental errors that cause these often deadly illnesses.

Human pluripotent stem cell research could also dramatically change the way we develop drugs and test them for safety. For example, new medications could be initially tested using human cell lines. Cell lines are currently used in this way (for example cancer cells). Pluripotent stem cells would allow testing in more cell types. This would not replace testing in whole animals and testing in human beings, but it would streamline the process of drug development. Only the drugs that are both safe and appear to have a beneficial effect in cell line testing would graduate to further testing in laboratory animals and human subjects.

Perhaps the most far-reaching potential application of human pluripotent stem cells is the generation of cells and tissue that could be used for so-called "cell therapies." Many diseases and disorders result from disruption of cellular function or destruction of tissues of the body. Today, donated organs and tissues are often used to replace ailing or destroyed tissue. Unfortunately, the number of people suffering from these disorders far outstrips the number of organs available for transplantation. Pluripotent stem cells, stimulated to develop into specialized cells, offer the possibility of a renewable source of replacement cells and tissue to treat a myriad of diseases, conditions, and disabilities including Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis and rheumatoid arthritis. There is almost no realm of medicine that might not be touched by this innovation. Some details of two of these examples follow.

- Transplant of healthy heart muscle cells could provide new hope for patients with chronic heart disease whose hearts can no longer pump adequately. The hope is to develop heart muscle cells from human pluripotent stem cells and transplant them into the failing heart muscle in order to augment the function of the failing heart. Preliminary work in mice and other animals has demonstrated that healthy heart muscle cells transplanted into the heart successfully repopulate the heart tissue and work together with the host cells. These experiments show that this type of transplantation is feasible.

- In the many individuals who suffer from Type I diabetes, the production of insulin by specialized pancreatic cells, called islet cells, is disrupted. There is evidence that transplantation of either the entire pancreas or isolated islet cells could mitigate the need for insulin injections. Islet cell lines derived from human pluripotent stem cells could be used for diabetes research and, ultimately, for transplantation.

While this research shows extraordinary promise, there is much to be done before we can realize

these innovations. Technological challenges remain before these discoveries can be incorporated into clinical practice. These challenges, though significant, are not insurmountable.

First, we must do the basic research to understand the cellular events that lead to cell specialization in the human, so that we can direct these pluripotent stem cells to become the type (s) of tissue needed for transplantation.

Second, before we can use these cells for transplantation, we must overcome the well-known problem of immune rejection. Because human pluripotent stem cells derived from embryos or fetal tissue would be genetically different from the recipient, future research would need to focus on modifying human pluripotent stem cells to minimize tissue incompatibility or to create tissue banks with the most common tissue-type profiles.

The use of somatic cell nuclear transfer (SCNT) would be another way to overcome the problem of tissue incompatibility for some patients. For example, consider a person with progressive heart failure. Using SCNT, the nucleus of virtually any somatic cell from that patient could be fused with a donor egg cell from which the nucleus had been removed. With proper stimulation the cell would develop into a blastocyst: cells from the inner cell mass could be taken to create a culture of pluripotent cells. These cells could then be stimulated to develop into heart muscle cells. Because the vast majority of genetic information is contained in the nucleus, these cells would be essentially identical genetically to the person with the failing heart. When these heart muscle cells were transplanted back into the patient, there would likely be no rejection and no need to expose the patient to immune-suppressing drugs, which can have toxic effects.

Adult Stem Cells

As noted earlier, multipotent stem cells can be found in some types of adult tissue. In fact, stem cells are needed to replenish the supply cells in our body that normally wear out. An example, which was mentioned previously, is the blood stem cell.

Multipotent stem cells have not been found for all types of adult tissue, but discoveries in this area of research are increasing. For example, until recently, it was thought that stem cells were not present in the adult nervous system, but, in recent years, neuronal stem cells have been isolated from the rat and mouse nervous systems. The experience in humans is more limited. In humans, neuronal stem cells have been isolated from fetal tissue and a kind of cell that may be a neuronal stem cell has been isolated from adult brain tissue that was surgically removed for the treatment of epilepsy.

Do adult stem cells have the same potential as pluripotent stem cells?

Until recently, there was little evidence in mammals that multipotent cells such as blood stem cells could change course and produce skin cells, liver cells or any cell other than a blood stem cell or a specific type of blood cell; however, research in animals is leading scientists to question this view.

In animals, it has been shown that some adult stem cells previously thought to be committed to the development of one line of specialized cells are able to develop into other types of specialized cells. For example, recent experiments in mice suggest that when neural stem cells were placed into the bone marrow, they appeared to produce a variety of blood cell types. In addition, studies with rats have indicated that stem cells found in the bone marrow were able to produce liver cells.

These exciting findings suggest that even after a stem cell has begun to specialize, the stem cell may, under certain conditions, be more flexible than first thought. At this time, demonstration of the flexibility of adult stem cells has been only observed in animals and limited to a few tissue types.

Why not just pursue research with adult stem cells?

Research on human adult stem cells suggests that these multipotent cells have great potential for use in both research and in the development of cell therapies. For example, there would be many advantages to using adult stem cells for transplantation. If we could isolate the adult stem cells from a patient, coax them to divide and direct their specialization and then transplant them back into the patient, it is unlikely that such cells would be rejected. The use of adult stem cells for such cell therapies would certainly reduce or even avoid the practice of using stem cells that were derived from human embryos or human fetal tissue, sources that trouble many people on ethical grounds.

While adult stem cells hold real promise, there are some significant limitations to what we may or may not be able to accomplish with them. First of all, stem cells from adults have not been isolated for all tissues of the body. Although many different kinds of multipotent stem cells have been identified, adult stem cells for all cell and tissue types have not yet been found in the adult human. For example, we have not located adult cardiac stem cells or adult pancreatic islet stem cells in humans.

Secondly, adult stem cells are often present in only minute quantities, are difficult to isolate and purify, and their numbers may decrease with age. For example, brain cells from adults that may be neuronal stem cells have only been obtained by removing a portion of the brain of epileptics, not a trivial procedure.

Any attempt to use stem cells from a patient's own body for treatment would require that stem cells would first have to be isolated from the patient and then grown in culture in sufficient numbers to obtain adequate quantities for treatment. For some acute disorders, there may not be enough time to grow enough cells to use for treatment. In other disorders, caused by a genetic defect, the genetic error would likely be present in the patient's stem cells. Cells from such a patient may not be appropriate for transplantation. There is evidence that stem cells from adults may have not have the same capacity to proliferate as younger cells do. In addition, adult stem cells may contain more DNA abnormalities, caused by exposure to daily living, including sunlight, toxins, and by expected errors made in DNA replication during the course of a lifetime. These potential weaknesses could limit the usefulness of adult stem cells.

Research on the early stages of cell specialization may not be possible with adult stem cells since they appear to be farther along the specialization pathway than pluripotent stem cells. In addition, one adult stem cell line may be able to form several, perhaps 3 or 4, tissue types, but there is no clear evidence that stem cells from adults, human or animal, are pluripotent. In fact, there is no evidence that adult stem cells have the broad potential characteristic of pluripotent stem cells. In order to determine the very best source of many of the specialized cells and tissues of the body for new treatments and even cures, it will be vitally important to study the developmental potential of adult stem cells and compare it to that of pluripotent stem cells.

Summary

Given the enormous promise of stem cells to the development of new therapies for the most devastating diseases, it is important to simultaneously pursue all lines of research. Science and scientists need to search for the very best sources of these cells. When they are identified, regardless of their sources, researchers will use them to pursue the development of new cell therapies.

The development of stem cell lines, both pluripotent and multipotent, that may produce many tissues of the human body is an important scientific breakthrough. It is not too unrealistic to say that this research has the potential to revolutionize the practice of medicine and improve the quality and length of life.

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[1] Michael Shamblott, *et al*, Derivation of pluripotent stem cells from cultured human primordial germ cells. *PNAS*, 95: 13726-13731, Nov. 1998.

James Thomson, *et al*, Embryonic stem cell lines derived from human blastocysts. *Science*, 282: 1145-1147, Nov. 6, 1998.